METHOD FOR SEPARATING ANALYTE FROM A SAMPLE

PARENT APPLICATION DATA

The application is a division of U.S. application Ser. No. 09/331,911, filed Jun. 25, 1999, now U.S. Pat. No. 6,440,725, which is a 371 of International Application PCT/US98/27632, filed Dec. 24, 1998, which international application is a continuation-in-part of U.S. Ser. No. 08/998,188, filed Dec. 24, 1997, now abandoned and is a continuation-in-part of U.S. Ser. No. 09/115,454, file Jul. 14, 1998, now abandoned, which is a continuation-in-part of U.S. Ser. No. 08/910,434, filed Aug. 13, 1997, now U.S. Pat. No. 6,368,871. All of these applications are incorporated by reference herein for all purposes.

FIELD OF THE INVENTION

This invention relates to a method for separating an analyte from a sample.

BACKGROUND OF THE INVENTION

The analysis of clinical or environmental fluids generally involves a series of chemical, optical, electrical, mechanical, or thermal processing steps on the fluid samples. Whether incorporated into a bench-top instrument, a disposable cartridge, or a combination of the two, such processing involves complex fluidic assemblies and processing algorithms.

Contemporary biomedical processing instruments are typically complex, robotically operated devices that move boluses of liquids automatically from one processing region to another. Prior cartridges have also generally processed a fluid sample as a fluid plug or bolus, moving a small quantity of sample from one region to another, where a further process is conducted. For example, Anderson et al. disclose such a device for sample processing in an article entitled "*Microfluidic Biochemical Analysis System*", Transducers '97, 1997 International Conference on Solid-State Sensors and Actuators, Chicago, Jun. 16–19, 1997, pg. 477–480.

In many analytical procedures, relatively large volumes of liquid (from microliters to milliliters) must be analyzed. Using the bolus approach, such volumes must be held in a container while each operation is performed. While the bolus approach allows for the implementation of complex processing methods, the volume of the fluid sample which can be processed is limited by the size of the individual processing regions, especially where the sample is transiently processed. Thus, the lowest detectable concentration of analyte, i.e. sensitivity, in any assay based on a bolus approach is also limited.

If the container is fabricated with integrated circuit chip technologies (microfluidic chips), the microfabricated chip must be very large to accommodate the relatively large volumes needed to detect a low concentration of analyte. For example, for a 100 microliter volume, a chip at least 1 cm on a side would be required for each bolus processing region. Such a large chip would not only be expensive, but would also defeat the purpose of miniaturization, especially for many types of disposable medical or environmental diagnostic uses.

Present day microfluidic technology has focused on picoliter, nanoliter, and microliter fluid volumes. These small volumes are not practical for many realistic diagnostic applications. As shown in FIG. **1**, the full range of chemical concentrations which one may want to detect in biological samples spans at least 20 orders of magnitude (from 6 copies/mL to 6×10^{20} copies/mL). Therefore, a cartridge for detecting the full range of potential analytes (especially DNA which exists in very low concentration in most biological samples) should be capable of processing large as well as small sample volumes.

Of special interest is the detection of low copy concentrations of analytes such as DNA, in which case large sample volumes are required. For example, the minimum theoretically detectable concentration for DNA probe assays necessitates large sample sizes, such as about 10^{-4} liters or more. In detecting infectious diseases, gram negative bacteria can be present at less than 10 copies per milliliter of blood, cryptosporidium generally appears as only a few copies per gallon of drinking water, concentrated biothreat agents, e.g. anthrax, at less than 100 copies per milliliter of water, and food poisoning agents, such as *E. coli* and salmonella, may be manifested in less than 10 copies per gram of food.

Thus, sample volumes needed to detect such infectious disease analytes would be larger than those required for detecting analytes present in higher concentrations, as in most clinical and immunochemistry assays. In addition, in the case of more concentrated analytes, such as those in immunoassays and clinical chemistry assays, a large volume sample provides more options for choosing less sensitive detection means, as well as the ability to divide the sample and detect multiple analytes. On the other hand, despite the merits of large sample volumes, it is generally recognized that unique functions can be realized with microfluidic structures, which are generally not compatible with large volumes.

SUMMARY

In a preferred embodiment, the invention provides a device for separating a desired analyte from a fluid sample and for concentrating the analyte into a volume of elution fluid smaller than the original sample volume. The desired analyte may comprise, e.g., organisms, cells, proteins, nucleic acid, carbohydrates, virus particles, bacterias, chemicals, or biochemicals. In a preferred use, the desired analyte comprises nucleic acid.

The device comprises a cartridge having formed therein an inlet port for introducing the sample into the cartridge and a sample flow path extending from the inlet port through the body of the cartridge. The sample flow path includes an analyte capture region having at least one flow-through component for capturing the desired analyte from the sample.

The flow-through component is preferably a microfabricated chip having a chamber with internal microstructures formed therein. The microstructures have sufficiently high surface area and binding affinity with the desired analyte to capture the analyte as the sample flows through the chip. The microstructures preferably comprise an array of columns integrally formed with at least one wall of the chamber and extending into the chamber. In an alternative embodiment, the flow-through component comprises a channel or chamber in the cartridge containing at least one solid support for capturing the analyte. Suitable solid supports include, e.g., filters, beads, fibers, membranes, glass wool, filter paper, polymers and gels.

A flow path for carrying elution fluid is also formed in the cartridge. The elution flow path passes through the flow-through component, thereby releasing captured analyte from the component into the elution fluid. The elution flow path diverges from the sample flow path after passing through the